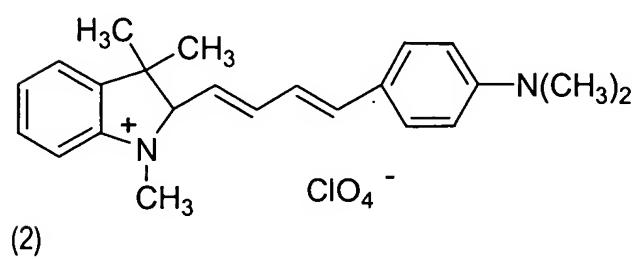


Listing of Claims:

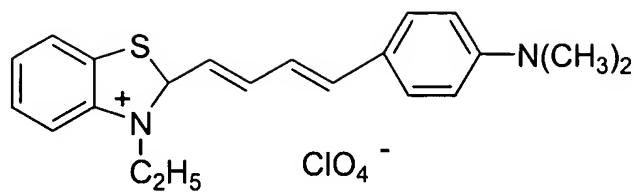
1. (Currently Amended) A method of staining bacteria comprising: ~~working~~ adding a polymethine dye ~~on~~ to a sample in the presence of a substance capable of reducing nitrite ions to stain bacteria in the sample.
2. (Original) A method according to claim 1, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, aspartic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfuric acid, pyrosulfuric acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.
3. (Currently Amended) A method according to claim 1, wherein the polymethine dye is at least one selected from the following group consisting of:

(1) Thiazole Orange;

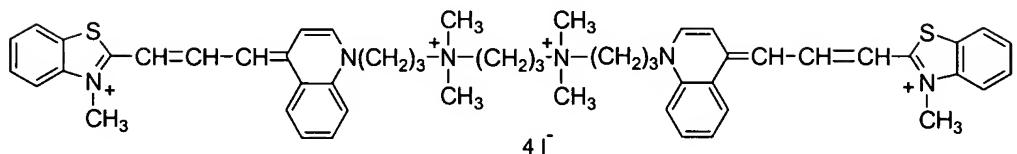
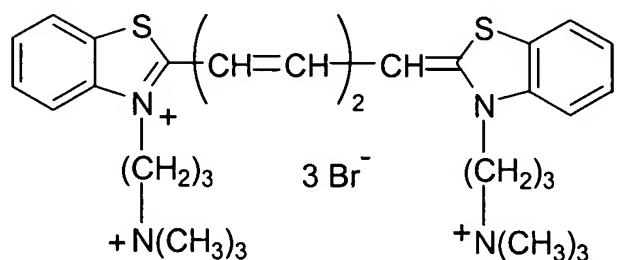


(2)

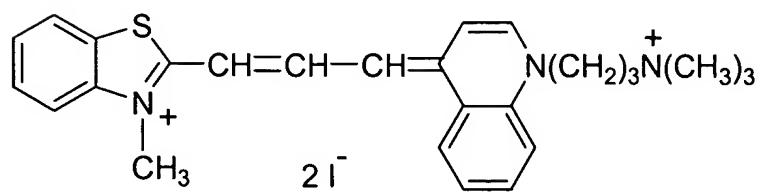
(3)

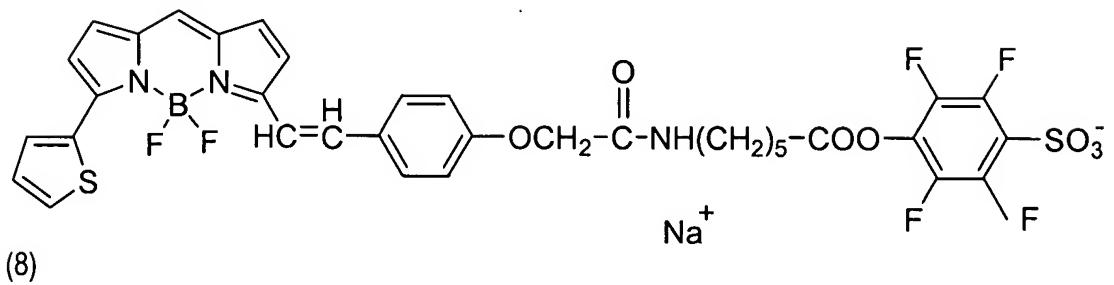
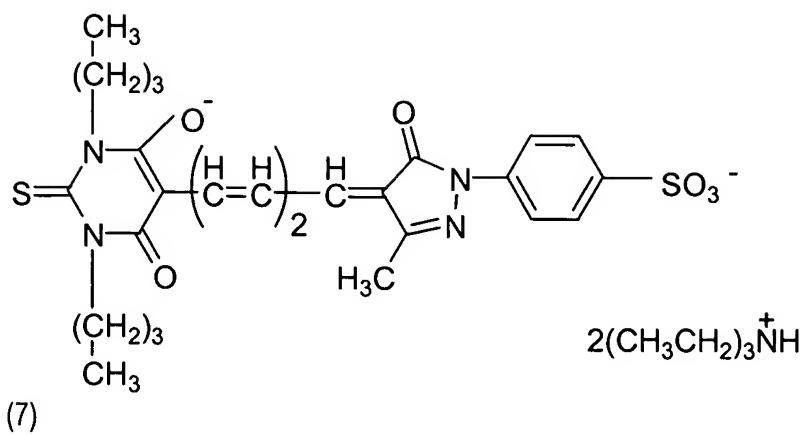


(4)

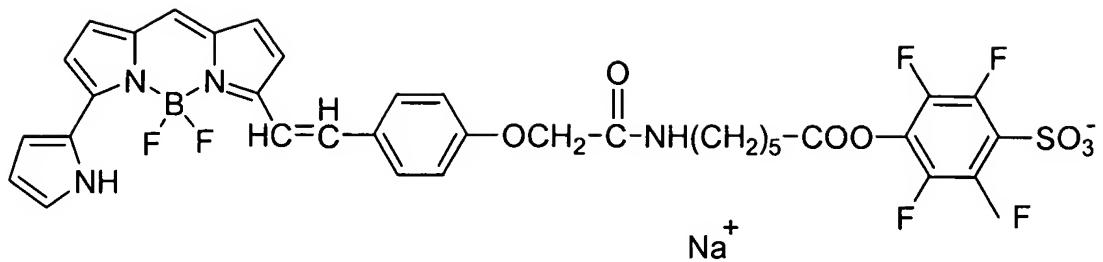


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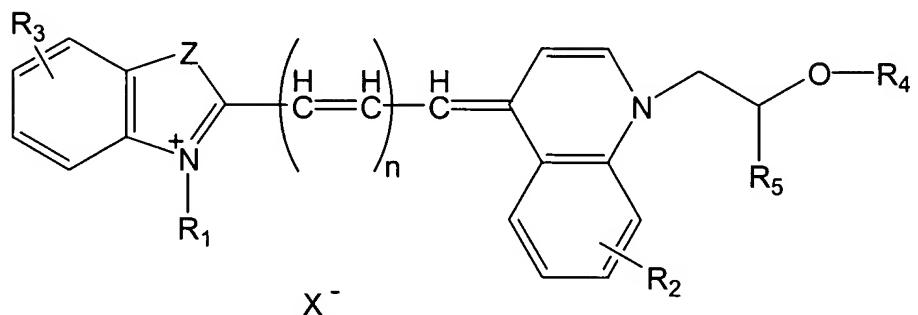




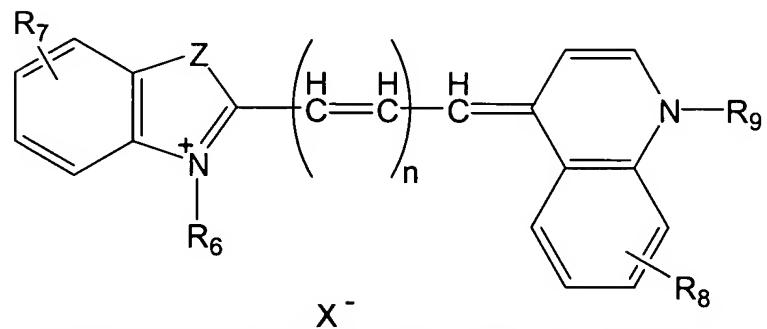
(9)



(10) a compound represented by the following general formula:



wherein R₁ is a hydrogen atom or a C₁₋₃ alkyl group; R₂ and R₃ are a hydrogen atom, a C₁₋₃ alkyl group or a C₁₋₃ alkoxy group; R₄ is a hydrogen atom, an acyl group or a C₁₋₃ alkyl group; R₅ is a hydrogen atom or a C₁₋₃ alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C₁₋₃ alkyl group; n is 1 or 2; X⁻ is an anion; and

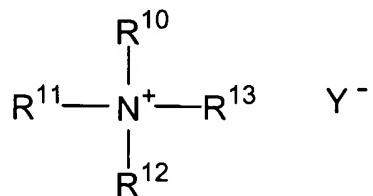


(11) a compound represented by the following general formula:

wherein R_4 R_6 is a hydrogen atom or a C_{1-18} alkyl group; R_2 R_7 and R_3 R_8 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 R_9 is a hydrogen atom, an acyl group or a C_{1-18} alkyl group; Z is sulfur, oxygen or carbon having a C_{1-3} alkyl group; n is 0, 1 or 2; X^- X is an anion.

4. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with presence~~ of a cationic surfactant.

5. (Currently Amended) A method according to claim 4, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R^{10} is a C_{6-18} alkyl group or $(C_6H_5)-CH_2-$; R^{11} , R^{12} and R^{13} , the same or different, are a C_{1-3} alkyl group or a benzyl group; Y^- is a halogen ion.

6. (Original) A method according to claim 5, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

7. (Currently Amended) A method according to claim 1, wherein the ~~dye is worked under sample is in an acidic state~~.

8. (Original) A method according to claim 7, wherein the acidic state is set at pH 2.0-4.5.

9. (Currently Amended) A method according to claim 4 Z , wherein a buffer of pKa 1-5.5 is used to maintain an acidic pH.

10. (Original) A method according to claim 9, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH, ϵ -aminocaproic acid-HCl, fumaric acid-HCl, β -alanine-NaOH and glycine-NaOH.

11. (Currently Amended) A method according to claim 1, ~~wherein the working is~~ carried out in the ~~existence with presence of~~ an inorganic salt of either sulfate or nitrate.

12. (Original) A method according to claim 1, wherein the dye is worked at 0.1 to 100 ppm in the sample.

13. (Currently Amended) A method according to claim 1, wherein the substance capable of reducing nitrite ions ~~exists is present~~ in the sample in such an amount that it can reduces the nitrite ions produced by bacteria of 10^5 to 10^8 /ml.

14. (Original) A method according to claim 1, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

15. (Original) A method according to claim 10, wherein the acid or the compound maintaining an acidic pH exists at 10 to 500 mM in the sample.

16. (Original) A method according to claim 1, wherein the sample is a urine, blood or spinal fluid.

17. (Currently Amended) A method of detecting and counting bacteria comprising the following steps of:

(1) ~~working adding~~ a polymethine dye ~~on to~~ a sample by a method as described in ~~any one of claims~~ claim 1 to stain bacteria in the sample,

(2) introducing the ~~thus~~ treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and

(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the number of the bacteria.

18. (Original) A method according to claim 17, wherein the step (1) is carried out by the steps of

(a) mixing a sample with an aqueous solution containing a substance capable of reducing nitrite ions and/or a cationic surfactant to accelerate dye transmissivity of bacteria;

(b) staining the bacteria for a predetermined period with a polymethine dye;

19. (Original) A method according to claim 17, wherein the step (3) of discriminating and counting the bacteria is carried out in accordance with at least one selected from the following combinations of:

(i) a forward scattered light intensity and a forward scattered light pulse width;

(ii) a forward scattered light intensity and a fluorescent light intensity; and

(iii) a forward scattered light pulse width and a fluorescent light intensity.